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HISTOLOGY OF THE OVIDUCT OF THE HEN.

This bulletin contains a detailed account of the microscopic anatomy of the oviduct of the domestic fowl. No such account has hitherto existed. The facts set forth in this bulletin are essential to an understanding of the physiology of albumen, membrane and shell formation in the making of an egg.

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BULLETIN No. 206.

THE HISTOLOGY OF THE OVIDUCT OF THE DOMESTIC HEN.*

FRANK M. SURFACE.

CONTENTS

INTRODUCTION.

More than half the weight of a normal hen's egg is elaborated by the oviduct. At least three very distinct substances are formed by it. These are the albumen, the shell membrane and the calcareous shell. The albumen may be further subdivided into the thick and the thin albumen, while the chalazæ are usually regarded as modifications of this same substance. The shell membrane consists of two layers, an inner rather delicate layer and an outer thicker layer. These layers are separated at the large end of the egg, forming between them the so-called

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air chamber. The shell consists of several layers of which at least three may be distinguished: (1) the inner or mamillary layer, consisting of minute conical deposits of calcareous material; (2) the middle spongy layer which is composed of a comparatively thick meshwork of fibers; (3) a delicate outer shell cuticle. In many eggs there is further deposited in the various shell layers, one or more coats of pigment.

This brief statement of the more important substances formed by the oviduct serves to show the complexity of the physiological processes which occur within this organ. For some time past, this laboratory has been engaged, among other things, in a study of these physiological processes. To form a basis for further experimental work it has seemed necessary to gain a more thorough knowledge of the histological structure of the organ with which we are dealing. The present study is thus chiefly morphological. Some discussion of the probable physiological significance of the elements described will be given in the latter part of the paper. In the main, however, these deductions must be supplemented and possibly corrected by later physiological experiments.

The hen's egg has been a classical object for the study of vertebrate embryology for the past century and a half. One would naturally expect that the formation of the egg itself would have attracted some attention during this long period. It is surprising, therefore, to find so few observations either on the physiology of the process or on the morphology of the organs concerned. A few of the more important papers on these subjects are mentioned in the following paragraphs.

The extensive work of Coste ('47 and '49) is one of the earliest studies of these processes. He gives quite accurate and detailed observations on the physiology of the oviduct. He gave us our most complete account of the processes attending ovulation. He also records the observation of an egg in the upper part of the isthmus of which the lower end was covered with membrane while the upper end was naked. He also observed the formation of the shell. The articles of Merkel v. Hemsbach ('51) and Landois ('65) are chiefly interesting on account of the uniqueness of their views regarding shell formation. They believed that the shell membrane was a direct transformation of the muscular layers of the isthmus. One of

the principal reasons for this view was the fact that the fibers of the membrane swell up and expand when treated with alkali and contract when immersed in acetic acid. In this action they appeared to them to resemble smooth muscle fibers. Nasse ('62) and Blasius ('67) pointed out the incorrectness of this view.

Landois ('65) further believed that the mamillary layer of the shell consisted of the uterine glands which were cast off with each egg and then regenerated. He says (p. 4) "Nach meinen Untersuchung der Entwickelungsgeschichte der Schale im Eileiter kann hierüber kein Zweifel mehr obwalten. Es sind die Drüsen der Uterinschleimhaut, welche sich auf der Faserschichte der Eischale angesetzt haben." This theory was an attempt to bring the avian uterus into agreement with the known action of the mammalian uterus. Blasius ('67) among others brought forward considerable evidence to refute this theory and to show that all the shell layers were secreted by the oviduct.

Nathusius ('68) believed that the egg membranes were organic tissues and that the shell and its membranes were derived from an elaboration of the yolk membranes in which the calcareous matter was deposited. Agassiz, Milne-Edwards, Blasius and others recorded observations which disproved this theory.

The most important of the older works on the histology of the avian oviduct is that of Sacchi ('87). She studied in considerable detail the histology of the oviduct in both the active and inactive states. Many of the results of the present study are not in accord with Sacchi's findings. These will be discussed at the proper places in the following paper. Loos ('81) has also contributed a most excellent paper on the structure of the avian and amphibian oviduct. He was the first to point out the tubular nature of the albumen glands in certain birds (crow) and showed there that these had definite openings into the lumen of the duct.

Giacomini ('93) has studied the histology of the oviduct in various birds, including the domestic hen. In certain respects this work is more thorough than any which precedes it. Quite recently Sturm ('10) has given an account of some of the more

important features of the histology of the hen's oviduct and reviews some of the literature. Weidenfeld ('05) has also studied the histology of the hen's oviduct. Reference to these and other papers will be made at the appropriate places in the text.

After the major portion of the present paper had been written, the writer received the recent paper by Béla ('10) relating to the structure and function of the oviduct in the domestic pigeon. Unfortunately this paper is published in Hungarian which the writer is unable to read. With the aid of a few German notes kindly supplied by Dr. Béla and a careful study of the figures the writer has been able to make out some of the more important conclusions of this excellent paper. References to these points have been included in the following paper.

Material and Methods.

The material used in the present investigation consisted of the oviducts of Barred Plymouth Rock hens. All oviducts were taken from freshly killed healthy birds. The birds were selected so as to obtain oviducts in various stages. from that of actively laying birds to that of birds which had not laid for several weeks. Since the oviduct of a laying hen is a rather large organ, it was necessary in most cases, to fix only small pieces of the regions desired. In doing this the oviduct was removed from the hen. cut open along one side and spread out on a dissecting board. A rough outline drawing (actual size) was then made showing the characteristic regions and their limits. Small pieces 5 to 10 mm. square were cut out at the points desired and their position accurately charted on the diagram. These small pieces were fixed, imbedded in paraffin and sectioned in the usual manner.

For fixation a variety of fluids were used, among which were Flemming's chromo-aceto-osmic mixtures, Gilson's mercuro-nitric solution, osmo-sublimate, sublimate-acetic and Zenker's fluid. The Flemming and Gilson mixtures gave uniformly the best results.

Sections were cut from 5 to 7 mikrons thick. All staining was done on the slide. The most useful stains employed were the following: Heidenhain's iron-alum-haematoxylin, Delafield's

haematoxylin, Kresylichtviolett, Ehrlich-Biondi, safranin and gentian violet. Of these Heidenhain's haematoxylin was by far the most useful.

It is a pleasure to acknowledge my indebtedness to Miss Maynie R. Curtis, assistant in this department, for attending to the details of fixing, cutting and staining the sections used in this work.

General Account of the Structure of the Oviduct.

The oviduct of a hen consists of five more or less clearly delimited regions. Beginning at the anterior end of the duct, these are ordinarily designated as (1) the funnel, (2) the albumen secreting portion, (3) the isthmus, (4) the uterus or shell gland and (5) the vagina. The funnel, known in anatomical terms as the *ostium tubae abdominale,* is a delicate thin walled portion lying immediately ventral to the ovary. Its anterior end is expanded into long lip like processes (fig. 453). The lips quickly converge to form a rather narrow, thin walled tube. In the functional oviduct the length of this tubular portion (funnel neck) is from 2 to 4 cms. The epithelium on the wall of the funnel is folded into rather low longitudinal spiral ridges.

These ridges, with more or less interruption are continuous throughout the oviduct (fig. 453). They are greatly increased in height in the lower portions of the duct. Besides these primary ridges the epithelium also forms smaller secondary folds. These secondary folds are best developed in the posterior portion of the funnel (cf. fig. 458). Their presence in other portions of the oviduct is somewhat obscured by the enormous development of the glands. As will be pointed out farther on these secondary folds bear important relations to the large glands.

The funnel region grades rather gradually into the albumen secreting portion. Figure 453 shows the region where this changes takes place. There is no difficulty in distinguishing between the two regions. In the albumen portion the walls are much thicker and the longitudinal ridges are very much higher (fig. 454). This albumen portion is the longest of the five divisions. Its average length is about 42 centimeters in the functional oviduct. The division between the albumen portion

and the isthmus is very sharply marked. As clearly shown in figure 455, the real limit of the two regions appears as a narrow line extending around the oviduct. However, for some distance on either side of this the character of the longitudinal ridges is quite different.

In general appearance the isthmus resembles the albumen portion except that the ridges are not quite so high and the walls are more contracted forming a narrower portion of the duct. The isthmus in the laying hen measures on the average, about 12 cms. in length. The distinctive function of the isthmus is to secrete the shell membrane.

The isthmus is continued without any clear line of demarcation into the expanded portion known as the uterus or shell gland. (fig. 456) As may be seen from figure 456, the character of the ridges is quite different in the uterus. They are more numerous and are greatly broken up by transverse and diagonal folding. The walls of the uterus are not so thick as those of the two last mentioned regions. The length of this organ is also about 12 cms. The lower end is bounded by the large sphincter muscle.

The vagina extends from this sphincter muscle to the cloacal wall. The walls of the vagina are very muscular. The epithelial folds are much smaller than in the preceding section. The length of the vagina is between 12 and 13 centimeters.

In a typical section of the oviduct wall, as for example the albumen secreting portion, we can distinguish the following seven layers of tissue (cf. fig. 462): (1) covering the outside of the duct there is a thin, serous, peritoneal membrane; (2) immediately inside of this is a layer of longitudinal muscle fibers. As was recently pointed out by Miss Curtis ('10) these muscle fibers arise from and are continuous with the musculature of the dorsal and ventral ligaments of the oviduct. (3) Inside of these muscles is a layer of connective tissue in which the larger blood vessels are found. (4) Next there is a layer of circular muscle fibers, and (5) inside of this an inner layer of connective tissue. (6) In all parts of the oviduct except the funnel and vagina there is next a thick layer of convoluted tubular glands, and (7) finally inside of this glandular layer is the epithelium of the duct.

With this brief survey of the general anatomy of the oviduct we may next turn to the details of its histology. In doing this it will be advantageous to deal separately with the different regions of the oviduct.

Histology of the Funnel Region.

The extreme anterior portion of the oviduct is expanded into a long slit like opening. The walls of this opening are known as the lips of the funnel. They are continuous with the mesenteries of the dorsal and ventral ligaments of the oviduct. From these expanded lips the oviduct quickly converges to a comparatively narrow tube (fig. 453). This latter tubular portion is known as the funnel neck. In the laying hen it varies from 2 to 4 cms. in length. The entire funnel portion is characterized by its very thin walls and its very low longitudinal ridges. On the outer edge of the funnel lips the ridges are only slightly raised. They gradually increase in height throughout the length of the funnel. At the transition from funnel to albumen portion they increase in height very rapidly. (Cf. fig. 453) The reason for this will be discussed in a later paragraph.

Figure 457 shows under low magnification a transverse section of the wall of the funnel lips. It is thus from the extreme anterior part of the oviduct. To show the thinness of the walls in this region as compared with other portions of the oviduct, it is interesting to compare this figure with figures 462, 468 and 469. These figures are all reproduced with the same magnification (i. e. x 30). From figure 457 we note the presence of the very low longitudinal ridges. The epithelium is further thrown into a number of secondary folds. The significance of these folds will be discussed shortly. From this same figure we may also note the presence of the outer layer of peritoneum and immediately above this a more or less definite layer of longitudinal muscles. Inside of this layer is the connective tissue with scattered bundles of muscle fibers. In this region of the oviduct the bundles of muscle fibers are scattered through the connective tissue and are not sharply separated into definite layers. Even here, however, the distinction between the outer longitudinal and the inner circular layer is fairly clear and can be traced in the figure. The inner layer at this point runs some-

what diagonally with respect to the long axis of the oviduct. A little farther down the duct the fibers in this layer run around the oviduct at right angles to its long axis, i. e., become circular muscles.*

The innermost layer of the oviduct is the epithelium. From it arise all the glands which have to do with the secretion of the various egg substances.

In its embryological development, this epithelial layer has an origin very different from the other layers of the oviduct. The *Anlage* of the oviduct, known as the Müllerian duct, arises as a thickening along the Wolffian body just ventral to the gonad. This Müllerian duct is at first a solid cord of cells. It later acquires a lumen and grows posteriorly until it connects with the cloaca. At the time of this posterior growth mesenchyme cells migrate in from the surrounding tissue and form a layer about the duct. From this layer of mesenchyme cells are developed all the outer layers of the oviduct i. e. the muscles and connective tissue. On the other hand the epithelium and its derivatives, i. e., all glandular structures, are formed from the walls of the old Müllerian duct. Corresponding to this difference in origin, the functions of the two sets of tissue are entirely different. The epithelium is entirely concerned with secretion and ciliary movement while, as would be expected, the derivatives of the mesenchyme are concerned with supporting and muscular functions.

We will defer the description of the minute anatomy of the epithelium until we have examined the general relation of the layers in other parts of the funnel region.

Figure 458 shows under low magnification a section from near the middle of the funnel. (Cf. fig. 453). The wall of the oviduct is of about the same thickness here as near the funnel mouth. (Fig. 457) The same layers of tissue are present as in figure 457, but the muscles are arranged in more definite layers. The most marked difference in the two sections is in the height and arrangement of the longitudinal ridges. It is clear that these ridges are very much better developed in the middle of the funnel than nearer the mouth. These longitudinal

* It is of importance to mention here that Sacchi says that the outer layer of muscles is circular and the inner layer longitudinal. This is certainly an error. The point will be discussed more thoroughly in a later section of this paper.

ridges gradually increase in height from the almost flat condition near the funnel mouth until they reach their greatest development in the albumen secreting region. (Cf. fig. 462). At the point of transition from funnel to albumen portion the increase in the height of the ridges is more sudden. (Fig. 453). The reason for this is seen if we compare figure 459 with figures 457 and 458. Figure 459 represents under medium magnification a section of the inner layer of the oviduct wall at the place where the funnel region passes into the albumen portion. In figures 457 and 458 we note that the cores of the longitudinal ridges are composed of connective tissue alone. In figure 459, however, new structures appear in this connective tissue core. These are sections of the tubular glands which will be described later. From this point the tubular glands increase rapidly in number and size towards the posterior region of the oviduct. Figure 459 shows only the inner layers of the oviduct. The outer muscular and connective tissue layers here do not differ materially from those shown in figure 458.

We may now return to an examination of the epithelium in the funnel region. Near the funnel mouth and over the upper portion of the funnel neck the epithelium consists of only a single layer of cells. These cells however, are of two distinct kinds: (1) ciliated cells and (2) non-ciliated gland cells. As shown in figures 457 and 458, the epithelium in this region is thrown into a large number of small secondary folds. Over the tops and sides of these folds there is a single layer of ciliated cells. At the bottom of the grooves thus formed occur groups of gland cells. Figure 460 shows one of these small glands together with the character of the epithelium over the folds. Figure 461 shows a larger and more typical gland from the same region. Both figures are from the same oviduct. As shown in the figures the ciliated cells are long and columnar in shape. The slightly oval nucleus lies well toward the outer distal end of the cell. Each cell bears a number of large, strong cilia.

The non-ciliated gland cells are shorter, and as shown in figure 461 are usually more irregular in shape. These gland cells in the active oviduct stain much deeper than the ciliated cells. Especially is this true of the nuclei, many of which take a very dark haematoxylin stain. Figures 460 and 461 are both

drawn from an oviduct which was actively secreting albumen at the time the bird was killed. At that time there was an egg in the albumen secreting portion about 8 cm. from the funnel mouth. Consequently we may suppose that these gland cells had been emptied of their contents very recently. In birds which have not laid for a day or two these cells take a much fainter stain.

It will further be noted from figure 460 and 461 that the nuclei of these gland cells lie well towards the inner or proximal end of the cells. On the average these nuclei are considerably larger than those of the ciliated cells. This is perhaps partly accounted for by the larger volume of many of the gland cells.

The shape of these invaginated glands varies considerably. Probably the chief factor in determining their shape is the mechanical one connected with the growth and the folding of the epithelium. However, the type of gland shown in figure 461 is much more common than any other. On the lateral sides of the gland the cells are much shorter while over the central portion they are larger, forming a kind of hump in the center of the gland pouch. The cells in this central region are nearly the same length as the ciliated cells in the adjoining ridges. These central cells also have much larger nuclei than the cells nearer the sides of the pouch. As noted above this is probably due to their larger volume. It also frequently happens in this type of gland that one or the other side the gland grows out into a rather long pouch or tube. This point is of interest in connection with the development of the tubular glands which will be described in the next section.

Glands with other shapes also occur. One of these is shown in figure 460. No evidence is at hand to show that these latter glands differ from the ordinary type (fig. 461) in their histological structure.

So far as I know all previous students of avian oviduct histology have stated that there are no glands in the funnel. Thus Sacchi says in describing the funnel (p. 297) "mancano affatto nelle pieghe le cellule di secrezione." Sturm ('10) apparently on the authority of Weidenfeld ('05) says "Der Trichter sezerniert nicht." With this conclusion I must, most emphatically, disagree. The cells described in the preceding paragraph have every appearance of being gland cells. Further they are

found well out on the lips of the funnel. Figures 460 and 461 are both from sections of the funnel lips. The fact that there is no mass of glandular tissue beneath the epithelium such as occurs in other parts of the oviduct has probably led to the conclusion that there were no glandular structures present. However, as will be shown farther on in this paper, the glands found in the funnel region are very probably homologous with the larger glands occurring in other portions of the oviduct.

Béla ('10) evidently did not find any gland cells in the funnel region in the pigeon. His figures 1 and 2 fail to show any evidence of gland cells, although the character of the folds shown in his figure 1 is very similar to the condition found in the hen, (cf. my figure 457).

As we pass posteriorly along the funnel from the mouth the character of the epithelium changes in many respects. We may next examine a section from near the middle of the funnel, i. e., corresponding to figure 458. Figure 463 shows a portion of this epithelium under medium magnification. As stated before, the longitudinal ridges of the oviduct are much better developed here than nearer the mouth. The secondary folds of the epithelium are present as before. Collections of gland cells very similar to those found nearer the mouth of the funnel occur at the bottom of the grooves. The chief difference here is in the character of the epithelium which covers the top and sides of the secondary folds. Figure 463 shows a section through the middle of one of these folds with a glandular groove on either side. Portions of the adjoining folds show at the edges of the figure. The similarity of the glands to those described for the funnel mouth (fig. 461) is at once evident. As before short cells are arranged along the lateral edges of the groove, while longer cells form a hump in the center of the gland pouch. One difference is to be noted here, however. In the region of the longer gland cells, there is often a double row of nuclei. The row of nuclei nearer the outer or proximal end of the cells correspond in position and appearance to the gland cells on either side of the groove. The second row of nuclei lie much nearer the periphery of the cells. Further examination shows that the cells in which these latter nuclei occur, i. e., those nearer the periphery, are ciliated. Between these ciliated cells occur the non-ciliated gland cells in which nuclei lie well towards the inner end of the cell.

Turning now to a consideration of the cells covering the tops and sides of these folds, a condition quite different from that near the mouth of the funnel is found. In the first place the shape of the folds, while often very irregular. (Cf. fig. 458) are in general roughly triangular. (Fig. 463) This makes rather deep pouches at the sides of the glandular grooves. It will be remembered that near the mouth, the epithelium over these folds consisted of a single layer of rather long ciliated cells. Only a single row of nuclei was present (Cf. figs. 460 and 461). Here, however, the condition is more complicated. Figure 463 shows that the nuclei are arranged more or less irregularly but that several rows are present. Careful examination shows that not all of these cells are ciliated. In none of the preparation of this region which I have been able to obtain, do the cell boundaries come out distinctly. It is thus difficult to tell which cells are ciliated and which are not. By the examination of occasional sections in which some of the cell outlines are clear, it seems practically certain that the cells in which the nuclei lie well towards the periphery are ciliated. The cells with deeper lying nuclei on the other hand are non-ciliated gland cells. Except at the time these latter cells are actively discharging secretion their peripheral portion is very much compressed between the broader ciliated cells. This makes it very difficult to obtain good views of entire gland cells. Details regarding these epithelial gland cells will be discussed later in connection with other regions of the oviduct, which are more favorable for their observation.

When sections from this region of an active oviduct are stained with gentian violet or safranin the openings of the single celled glands described in the last paragraph take a much deeper stain than any other part of section. By this means it is possible to study the distribution of these gland cells over the folds of the epithelium. Examination of such sections shows that the opening of the glands are in general much more numerous along the sides of the folds than over the middle. This is also borne out by a study of the distribution of the deeper lying nuclei.

Béla ('10) (figure 4, plate 1.) shows these unicellular epithelial glands in the funnel neck of the pigeon. So far as

the writer is aware this is the first time that such glands have been noted in the funnel neck of an avian oviduct.

We may pass now to a brief consideration of the epithelium in the region of the transition from funnel to albumen portion. Figure 459 shows under rather low magnification, a portion of the inner layers of the oviduct at this point. The most characteristic thing here is the appearance of sections of tubules lying in the connective tissue below the epithelium. This is the first appearance of what we may call the *tubular glands*. From this point posteriorly to the vagina they form the most important organ of secretion in the entire oviduct. At this point in the oviduct there are only a few tubules present. It thus presents an excellent place for the study of the origin of these structures, and of their relation to other glandular elements of the oviduct. Figure 464 shows a small portion of the epithelium from this region of the oviduct. This figure shows the region between two of the secondary folds of the epithelium and thus corresponds exactly to the glandular grooves found farther forward in the funnel. If we compare figure 464 with figures 460 and 461, the resemblance can be traced very easily. In the middle of figure 464 there is a group of ciliated cells with gland cells between (the lower row of gland cell nuclei can be seen here.) On either side of this ciliated area occur groups of shorter gland cells. No ciliated cells are present in these latter areas. On the left side in figure 464 this glandular area is greatly extended forming a long branched pouch of which only a portion is shown in the section represented. Now the central group of long cells corresponds to the group of long ciliated and glandular cells which form the raised portion in the center of the glandular groove in figure 462. The non-ciliated glandular areas on either side of this ciliated tuft correspond to the corners of the glandular groove in figure 461. In figure 464 it is seen that one of these corners is greatly extended and enlarged to form a long pouch or tube. If the series of sections, from which this figure has been drawn, is followed through, it is seen that the other corner of the groove is also extended into a tubular structure. These tubular structures are the beginnings of long convoluted tubular glands. These tubular glands form the most conspicuous part of the oviduct in the region between the funnel and vagina. They

reach their greatest development in the albumen portion where they are very much more numerous and very much longer than in the region under discussion. (Cf. fig. 462.)

From the above description, it is clear that the tubular glands are homologous, anatomically at least, with the glandular grooves in the funnel region. These collections of gland cells are present even in the region of the funnel mouth. (Cf. fig. 461) As we pass back through the funnel they are still present but become somewhat better developed, larger and more numerous. The tendency to pouch out at the corners becomes more marked the farther one gets from the mouth. Finally at the beginning of the albumen secreting region the pouching is greatly increased and the first of the so-called tubular glands are found. These glands will be discussed more in detail in connection with the albumen secreting portion. Also the discussion of their possible function is deferred until all the anatomical facts are in hand.

The character of the epithelium other than that of the tubular glands does not differ greatly from the epithelium in the middle of the funnel. The longitudinal ridges here are broader than farther forward in the funnel. This can be seen from figure 453 also by comparing figure 459 with figure 458, although these latter figures are drawn with different magnifications.

The chief cause of this increase in width is found in the greater space taken up by the tubular glands. For this reason the secondary folds of the epithelium are forced farther apart and hence do not appear so numerous. Further, the development of the tubular glands give relief to growth forces in a downward direction, rather than a lateral one, hence the secondary folds are not so clearly cut as in the earlier sections of the funnel (Cf. figs. 459 and 458).

Now, as seen from figure 453 almost as much of the epithelial surface in the middle of the funnel is taken up by the glandular grooves as by the ciliated folds. With the broadening of the ridges at the beginning of the albumen portion, the glandular pouches are pushed farther apart, and hence more surface is devoted to the ciliated epithelium. Considering the increased glandular surface formed by the tubules it seems probable that this latter increases much more rapidly than the space devoted to the ciliated epithelium.

Just as in the section from the middle of the funnel the so-called ciliated epithelium here also contains unicellular glands. The arrangement of these is similar to that of those previously described. In figure 464 the ciliated epithelium contains two or more rows of nuclei. As before the outer smaller nuclei belong to the ciliated cells. Between these latter occur the gland cells with deeper lying and usually larger nuclei. The special histological description of these cells will be given in connection with the albumen portion, where they also occur.

SUMMARY OF SECTION ON HISTOLOGY OF THE FUNNEL.

Six tissue layers are distinguished in the funnel walls. Two of these are muscular layers, an outer longitudinal and an inner circular layer. In the funnel region these layers consist for the most part, of scattered bundles of fibers. On either side of the inner layer of muscles is a layer of connective tissue. The lumen of the funnel is lined with ciliated, glandular epithelium. The outside of the oviduct is covered by a thin peritoneal layer.

The inner surface of the oviduct is thrown into a large number of primary longitudinal ridges. The epithelium over these ridges still further forms secondary folds. Three types of glands are described. These are (1) the unicellular glands occurring between the ciliated cells in the epithelium. These glands are not found in the anterior half of the funnel. (2) The glandular grooves. These are accumulations of gland cells at the bottom of the grooves between the secondary folds of the epithelium. These are found in all portions of the funnel region except in the extreme posterior part. Here they are replaced by (3) the tubular glands. These latter glands are undoubtedly homologous with the glandular grooves found farther forward.

HISTOLOGY OF THE ALBUMEN SECRETING REGION.

That part of the oviduct lying between the funnel and the isthmus is ordinarily designated as the "albumen secreting portion." While there is good reason to believe that the secretion of albumen is not confined to this region alone, nevertheless it is here that the major portion of this substance is formed.

In the active oviduct of a Barred Plymouth Rock hen the albumen portion measures, on the average, nearly 42 cm. in length, or more than half the total length of the oviduct.

As shown in figure 454, the longitudinal ridges are very high and broad in this part of the oviduct. A better idea of the development of these ridges can be obtained from figure 462. This figure is drawn with the same magnification as figures 457, 458, 468 and 469. It is seen at once that these ridges are much higher and broader here than in any other part of the oviduct. The major portion of this increase in the size of the ridges is due to the greater development of the tubular glands. These glands form a distinct tissue layer between the inner layer of connective tissue and the epithelium.

The muscular layers in the albumen region are somewhat thicker and the bundles are much better segregated into definite layers than in the funnel region. However, the musculature of this region is much less developed than in the parts of the oviduct posterior to it. This point is clearly seen by comparing figure 462 with figures 468, 469 and 473. The slow movement of the egg through this part of the duct is evidently associated with its weak musculature.

As shown in transverse section (figure 462) the fibers in the outer layers of muscles, run in general in a longitudinal direction through the oviduct, but at the same time they take a slightly spiral course. That the inner muscular layer runs in a circular direction is evident from figure 462. These sections have been cut very nearly transverse to the long axis of the oviduct. Consequently the long fibers seen in the inner muscular layer certainly run in a transverse or circular direction. The spiral course of the fibers in the outer muscular layer rather obscures the fact as to their essentially longitudinal nature. These same facts have been brought out by Miss Curtis ('10) in connection with the study of the ligaments of the oviduct. She has shown by means of very careful dissections that the outer muscular layer, which is continuous with the musculature of the ligaments, is longitudinal and that the inner layer consists of circular fibers. These facts are opposed to Sacchi's statement that the internal muscular layer is longitudinal and the outer circular. Sacchi says (p. 297) in regard to the funnel region, "L'epithelio che le ricopere è pure cylindrico e munito di ciglia; il connessivo è attraversato da vasi, e verso l 'esterno vi sono frammisti fasci muscolari longitudinali isolati, circondati da muscoli circolari e poi dal connessivo

esterno." A similar statement regarding the musculature of the uterus is made on pages 299-300. There is no doubt but that Sacchi is wrong as to the essential nature of these muscular layers.

Since it is in this region of the oviduct that the tubular glands reach their greatest development, it is thus a proper place to enter into a detailed description of their form and histology. The method of origin of these glands and their homology with certain epithelial structures of the funnel has already been pointed out (pp. 407-408). It has also been noted that the invaginated tubules follow very tortuous courses and often branch and rebranch so that it is very difficult to follow the course of a single tubule. In point of fact it is much easier to follow a tubule at the beginning of the albumen portion than at any other place in the oviduct. In the region of the oviduct represented by figure 462, it is very difficult to follow individual tubules. For the purpose of learning something about the length and form of the tubules, several individual glands have been traced for some distance by making camera drawings from a series of sections. The only adequate method of representing these tubules would be by wax plate reconstructions. The point in question, however, has not been deemed worth the time and labor required to make such a model. Figure 462 shows that there is a very large mass of tubules present. Examination of the sections shows that there are relatively few openings of these tubules into the lumen of the duct. From this evidence alone, we might conclude that each tubule must be of considerable length and consequently convoluted.

The openings of the tubules are more numerous along the sides and near the bottom of the large ridges. The positions of these openings are marked by small indentations of the epithelium. Several of these indentations can be seen in figure 462. These invaginations of the epithelium are of the nature of short ducts which connect with the lumen of the tubule. Figure 465 shows the opening of one of these tubules in the albumen region. In many cases several tubules open through the same duct. Such a case is shown in figure 470 from the isthmus region.

The tubular nature of these albumen secreting glands, and especially the presence of openings into the lumen of the ovi-

duct, seems never to have been clearly recognized in the case of the domestic fowl. Loos ('81) describes this condition very clearly for the oviduct of the crow (*Corvus corone*). He states that at the beginning of the breeding season the epithelium of the oviduct invaginates at many places. These invaginations grow deeper into the connective tissue and form a tube. After this tube has reached the connective tissue septum it bends and grows in the opposite direction. These tubes often branch or follow a winding course. With regard to the fowl's oviduct, Loos is not so clear. Other writers and particularly Sacchi ('88) fail to recognize the openings of the tubules into the oviduct. Gadow ('91) in Bronn's "Tierreichs," who follows Sacchi chiefly, makes the following statement (p. 845) "In jede Falte (of the mucosa) erstreckt sich ein centraler Stamm von Bindegewebe der Submucosa, welches sich dann peripherisch verästelt. Der Raum zwischen diesen Verästelungen ist mit zahlreichen, unregelmässig gelagerten, polyhedrischen Zellen erfüllt welche zwischen sich kleine Massen von Eiweiss absondern. Die Oberfläche der Falten trägt überall hohe sehr regelmässige Cylinderzellen, durch welche dann die in der Tiefe secernirten Eiweissklümpchen austreten und um die Eikugel abgelagert werden."

This statement which Sacchi also makes, viz., that the secretion from the deeper lying gland cells passes out through the epithelial cells, is most certainly in error. Further the gland cells beneath the epithelium are not "unregelmässig gelagert," but are arranged very definitely into tubules as shown in figures 465, 471 and 472. Sacchi very probably saw the unicellular glands in the epithelium and believed that these were ducts passing the secretion from the deeper cells rather than being themselves secretory.

Going back to the description of the tubules, we note from figure 465, that the lumen of the tubules are filled with a finely granular secretion. Further, the cells which form the walls of the tubules are also distended with a granular substance. Within the cell the granules vary in size from those barely visible, and perhaps smaller, up to a few rather large granules. This represents the condition in that portion of an oviduct which is just beginning to pour out its secretion. Figure 465 is drawn from a section of the albumen region taken a few centimeters

in front of an advancing yolk. The small, very dark staining nuclei in these sections should be noted.

If we examine a section of the oviduct which has just passed the active period of secretion we find the protoplasm still very finely granular. It now has an alveolar appearance. This is shown in figure 471 which is taken from the isthmus. Similar appearances may also be found in the albumen region.

If sections are cut from a region of the oviduct which would not normally secrete for several hours, the appearance of the cells is somewhat different. Here, as shown in figure 466, each cell contains several very large granules which take an intense haematoxylin stain. Around each of these granules is a light area which gives the appearance of a solution zone. The remainder of the cell cytoplasm is finely granular and often presents an alveolar appearance. (cf. fig. 466). It should be said that I have never been able to obtain sections which show the condition just described (fig. 466) throughout the section. In all cases the first three or four cell layers beneath the epithelium present this appearance in great detail. Beyond this the granules begin to fade out until those cells towards the center of the ridges show no evidence of granules. I have attributed this condition to the action of the fixing fluids used.

No attempt has been made to study in detail the origin and growth of the gland granules. The observations reported in the above paragraphs have been made in the course of the study of the grosser histology. In general I think the changes undergone by the granules in these glands can be said to resemble in many ways the changes described by Langley, E. Müller and others for the granules of the salivary and digestive glands.

As shown in figure 462, there is a layer of connective tissue between the inner layer of muscles and the layer of tubular glands. This connective tissue extends into the center of each ridge forming a core. At the same time the connective tissue cells are found along the walls of the tubules and often form a thin layer between the epithelium and the tubules beneath it. In figure 465 the gland cells are so distended with secretion that very few connective tissue cells can be seen. In figures 470 and 471, from the isthmus these cells are very evident. The same conditions are found in the albumen region. The connective tissue carries with it of course the smaller blood vessels. Ex-

amination of the sections show small capillaries running everywhere between the tubules.

We may next turn to a discussion of the epithelium. As stated on page 401, the epithelium of the funnel region consists of simple, ciliated, columnar cells. In all other portions of the oviduct however, there are two kinds of cells present in the epithelium. These are the ciliated cells and alternating with these are non-ciliated unicellular glands. So far as I know no one has hitherto pointed out the presence of these latter gland cells in the fowl's oviduct. However, with good preparations there cannot be the slightest doubt of their presence. Béla ('10) describes and figures these goblet cells in the oviduct of *Columba,* and Giacomini ('93) also described such mucine glands in the oviduct of certain birds. Figures 465 and 467 show the epithelial portion of an actively secreting oviduct. The distribution of these two kinds of cells is fairly even over the entire inner surface of the oviduct. In general the ciliated cells and the gland cells appear to alternate. At the point of invagination of the epithelium to form the short ducts to the tubules the unicellular glands appear larger and perhaps more numerous. This is shown somewhat indistinctly in figure 465. Many sections show this point more clearly.

The ciliated cells are of the usual columnar type. Their general appearance can be seen in figure 467. They consist of finely granular protoplasm with a rather large, usually oval nucleus. The nucleus nearly always lies about the middle of the cell or slightly towards its distal end. The distal end of the cell is armed with a large number of strong cilia. As shown in figure 465 and 467, these cells are much crowded out of shape by the great development of the gland cells. In figures 470 and 471 the gland cells are less distended and the ciliated cells present a more normal appearance.

The epithelial gland cells are of the goblet (Becherzellen) type. As stated above, they alternate with the ciliated cells. The proximal portion of the cell in the actively secreting oviduct is finely granular while the more distal portion presents an alveolar structure. Whether the alveoli represent the gland granules or an intergranular network, I cannot say. These cells as shown in figures 465 and 467 have all the appearance of being distended with secretion. In most cases they have

crowded the ciliated cells to one side and have extended distally beyond the former limit of the epithelium. This is the stage immediately preceding secretion. In sections taken from an oviduct at the point where a yolk was descending, the secretion from these glands can often be seen pouring out in little streams from each cell. The nuclei of the gland cells are as a rule more nearly circular in outline than the nuclei of the ciliated cells. Further a very characteristic difference between the two kinds of cells is found in the position of the nucleus. The nuclei of the gland cells lie well towards the proximal ends of the cells, while, as stated above, the ciliated cells have their nuclei towards the distal ends. (Cf. fig. 465, 467, 470, and 471).

One point should be mentioned in regard to technique in studying these epithelial glands. While it is possible to demonstrate the presence of these glands in the sections of every active oviduct which I have examined, they do not show with the same distinctness in all cases. When these glands are not filled with secretion they are crowded back by the then larger ciliated cells. In such cases casual examination of the epithelium shows an almost completely ciliated surface. However the double row of nuclei are present and more careful study of the section has never failed to show the glands themselves. Further it is possible to demonstrate their presence by methods of differential staining as given in a later paragraph. If, however, sections are taken from a region where these glands are filled with secretion, they form the most conspicuous part of the epithelium. The sections which show these glands best have been obtained from oviducts in which the yolks had just passed the funnel region. In such an oviduct the albumen region is just on the point of extruding its secretion (cf. figs. 465 and 467).

The question now arises as to whether the tubular glands, which are derivatives of the epithelium, differ in the character of their secretion from the goblet glands in the epithelium itself. It is conceivable of course, that the two sets of glands are really concerned in the secretion of the same substance, the only difference being that the invaginated portions have lost the ciliated cells. On the other hand it is conceivable that the two sets of glands secrete entirely different substances, and that a proper mixture of the two are necessary to form the albumen as we know it.

While the final proof as to the identity on non-identity of the products of these glands must come from physiological experiments yet some evidence as to its nature can be gained from the morphology of the glands themselves. In the first place the appearance of the cytoplasm in the two sets of glands is different in all the sections which I have examined. In the tubular glands the protoplasm always consists of much coarser granules than are ever found in the epithelial cells. The most important difference, however, is in their micro-chemical reactions to various stains. Very few of the stains which I have used stain the contents of these two sets of glands with the same intensity. In the case of Heidenhain's iron-alum-haematoxylin alone, the cells of the tubular glands always take a much darker stain. The epithelial gland cells show a very slight haematoxylin stain, unless the whole section is very deeply colored.. Instead the epithelial cells have a yellow appearance with only the very small granules dark colored. If Bordeaux red is used as a counter stain this colors the cytoplasm of the tubular glands but unless very deeply stained it does not affect the epithelial glands at all. In Ehrlich-Biondi stain the granules of the tubules take a dark brownish red color while the granules of the epithelium are a yellowish red. In Kresylichtviolett the unicellular glands stain very dark purple and stand out plainly between the ciliated cells. On the other hand the tubular glands stain a very light violet. Likewise with safranin the unicellular glands take a very much deeper stain. The evidence at hand thus points to a difference in the function of these two sets of glands. The possible significance of these facts will be discussed in another place. (Cf. pp. 424-425).

SUMMARY OF SECTION ON ALBUMEN REGION.

The bundles of muscular fibers are more sharply segregated into definite layers than in the funnel. Compared with more posterior regions of the oviduct the musculature of this portion is very poorly developed. Evidence is given to show that contrary to the statement of Sacchi and others, the outer muscular layer consists of longitudinal fibers while the inner layer contains circular fibers.

The walls of the albumen region are much thicker than those of other parts of the oviduct. This increase in thickness is due

to the great development of the tubular glands. These glands are formed by invagination from the epithelium. Each gland is a long, convoluted and branched tubule which opens to the lumen of the oviduct by means of a short duct. The cells of these tubular glands are characterized by small very dark staining nuclei which lie close to the basal walls of the cells. The protoplasm is very granular especially before secretion. After being emptied of its secretion, the cell has a more alveolar appearance.

The epithelium consists of two kinds of cells; (a) Ciliated cells and alternating with these (b) unicellular glands. Difference in the appearance and staining reactions indicate that these unicellular glands secrete a different substance from that produced by the cells of the tubular glands.

Histology of the Isthmus.

As stated on page 400 there is a very sharp line of demarcation between the albumen region and the isthmus. Towards the posterior end of the albumen region the longitudinal ridges become much lower. (Fig. 455). On the isthmus side the ridges are also quite low for two or three centimeters. After that the ridges in the isthmus gradually become higher although they never reach the height or breadth found in the albumen portion. The real division between these parts of the oviduct is a narrow line extending entirely around the duct. It is plainly visible in the fresh oviduct as a line of translucent tissue. (Fig. 455.)

If we cut sections in such a way as to go through this division line, we find the reason for its appearance. Figure 469, represents such a section. The albumen region is represented at the left of the figure and the isthmus at the right. It is seen at once that between these two regions the tubular glands are either entirely or partially lacking. It is this feature which causes the appearance of the line on the oviduct. The large mass of tubular glands in the ridges is replaced by connective tissue. The epithelium presents the same appearance as in the albumen portion. Both the ciliated cells and the epithelial glands are present.

It should be said that the section represented in figure 469 is cut very obliquely to the long axis of the oviduct. It thus hap-

pens that the muscular layers appear very much as in the transverse sections.

The histological structure of the isthmus proper is practically the same as that of the albumen region. Both sets of glands are present and have practically the same microscopic appearance as in the albumen portion. Figure 470 is drawn from a section in the middle of the isthmus. It will be noted that in the epithelium both the ciliated cells and the unicellular glands are present. These have the same appearance as in the albumen region in a similar stage of physiological activity.

The tubular glands do not form so thick a tissue layer here as in the albumen portion but in their microscopic appearance they are very similar in the two regions. From figure 470 we note the presence of the dark irregular nuclei lying near the basal walls of the cells. The cytoplasm in this section presents a somewhat more alveolar appearance than is shown in the figures from the albumen region. This is not a constant difference. Either the granular or alveolar appearance may be found in either region depending perhaps on the state of physiological activity and also possibly upon the method of fixation.

Although not shown in the figures it may be stated that the muscular layers, particularly the circular muscles, are somewhat thicker and better developed in the isthmus than in the albumen region. Corresponding to this the walls are firmer and more constricted than in the albumen region. This is no doubt associated with the shorter length of time required for the egg to traverse the isthmus.

It will be remembered that the distinctive function of the isthmus is to secrete the shell membrane (*Membrana testacea*). We have seen, however, that this region presents no visible differentiation which does not also occur in the albumen secreting portion of the duct. This similarity in structure does not necessarily imply a similarity in physiological activity. It is quite conceivable that the glands in the isthmus secrete different substances from the similar glands in the upper portion of the oviduct. This question will be discussed more in detail after we have examined the structure of the uterus and vagina.

HISTOLOGY OF THE UTERUS.

There is no clear dividing line between the isthmus and the uterus such as was described between the albumen portion and the isthmus (p. 400). Instead the isthmus passes gradually into the expanded uterus without any distinct break in the tissue layers. As is clearly shown in figure 456 the character of the longitudinal ridges in the uterus is quite different from that of the homologous structures in any other portion of the duct—in fact the ridges, as such, are lost and instead we find small leaf like folds of the epithelium. The immediate cause of this change in the character of the folds is probably a mechanical one connected with the greater amount of surface to be covered. If the same number (20 to 30) of ridges which are found in the albumen portion and the isthmus were continued over the expanded uterus they would lie rather far apart in this latter region. The branching of the ridges and the refolding of the intervening surface would account in some measure for the change in the character of the folds. There may also be some physiological reasons for this change since as we shall see the character of some of the glands in the uterus differ from what has been found in the other portions of the duct.

As shown in figure 468 the same muscle layers are present in the uterus as in the more anterior regions. These layers, particularly the outer, longitudinal muscles, are somewhat thicker and better developed than in the regions previously discussed. However, there is but little difference in this respect between the isthmus and the uterus. The connective tissue layers present no change of any importance from the conditions found in the anterior regions. Under low magnification the cross section of the epithelial folds in the uterus do not differ essentially in appearance from those found in the isthmus. The folds, however, are not so high and perhaps on the average contain a thicker layer of glandular tissue (tubular glands). The epithelium covering these ridges presents the same general appearance as that found in the isthmus and albumen portion. There is then no striking morphological differentiation in the appearance of the tissue layers of the uterus which would indicate its specific function of shell formation. The same layer of tubular glands and as we shall see, the same ciliated, glandular epithelium occurs here as elsewhere. If any morphological

differentiation is to be found it must be looked for in the minute histology of the glands.

Under higher magnification it is clearly seen that the layer of gland tissue is composed of tubules very similar in shape and size to those seen in the earlier sections. Further search reveals the openings of these gland tubules through the epithelium (fig. 472). The openings of these glands appear to differ somewhat from those found in the anterior regions, e. g. in the albumen portion (cf. fig. 465). In the albumen region it appears as if the epithelium were invaginated to meet the gland tubules while in the uterus the gland cells appear to force their way through the epithelium with only a slight indentation of the latter. In the one case a short epithelial duct is formed, while in the other, the duct, if it may be so called, is composed of gland cells. I am not at all certain that any significance should be attached to this point but I have observed this difference very constantly in my sections.

The chief difference in the appearance of the uterine glands is in the appearance of the gland cells themselves. A comparison of figures 465 and 472 will make clear this difference between the albumen and uterine regions. Both figures are taken from active oviducts. In the active gland from the albumen region the nucleus is small, dark staining and lies well towards the proximal end of the cell. In the uterus the cell nucleus is larger, more regular in shape and usually lies towards the center of the cell. The chromatin granules and nucleoli take on a comparatively deep haematoxylin stain but the nucleus as a whole does not show the intense stain found in the albumen and isthmus regions.

The cytoplasm in cells of the uterine tubular glands does not present the heavy granular appearance described for the albumen portion. Instead these cells are diffusely granular in appearance. The granules are all of small size and take a rather weak stain with haematoxylin. In some cells there is evidence of the clumping of these granules, especially towards the periphery of the cell. I am not sure that this is not an artifact due to the fixation.

In actively secreting glands (fig. 472) this granular material can be seen lying outside the gland cells in the ducts and even in the lumen of the oviduct itself.

Turning now to the epithelium in the uterine region we find, as in the anterior parts of the oviduct, both the ciliated cells and the unicellular glands. These present practically the same arrangement and appearance as in the previous sections. Except at their distal ends the cell boundaries of these epithelial cells are very difficult if not impossible to see. The basal portion of the epithelium appears as a syncytium. This appearance may be due to the technique employed.

From the above description and figures it is clear that in the uterus the cells of the tubular glands have a somewhat different structure from the cells found in these glands in other regions of the duct. This difference in structure is undoubtedly associated with the particular function of shell secretion which is confined to the uterus.

Histology of the Vagina.

The posterior end of the uterus is marked by the strong sphincter muscle. The contracted portion of the oviduct extending from this sphincter muscle to the cloacal wall is known as the vagina. As shown in figure 456 its walls are much contracted. The inner surface is thrown into rather low longitudinal ridges which appear in general to be continuous with the folds in the uterus (fig. 456). The length of the vagina is, on the average, about 12 cm. in the laying hen.

Figure 473 represents a cross section of the wall of the vagina under low magnification. The most striking character in this figure is the great development of the inner (circular) muscular layer. In the vagina this layer is several times thicker than in any other region of the oviduct. The great development of this muscular layer is naturally associated with the need of expelling the egg which has acquired its full size and is now a firm object upon which the muscles can act. The outer longitudinal muscular layer consists of bundles of fibers scattered through the connective tissue. Compared with the circular layer the longitudinal muscles are but poorly developed. The connective tissue layers are similar to those in other parts of the duct.

As shown in figure 473, the longitudinal ridges in the vagina region are low and rather narrow. The cores of these ridges are filled with connective tissue and no layer of tubular glands is

present. The epithelium covering these ridges is thrown into a large number of secondary folds.

In figure 474, two of the secondary folds from the epithelium of the vagina are shown under higher magnification. From this figure we note:

1. The entire absence of any structures resembling the tubular glands. The space beneath the epithelium is occupied by connective tissue (cf. fig. 473).

2. As in other parts of the oviduct, excepting the funnel, two kinds of cells occur in the epithelium, viz: the ciliated cells and the unicellular glands.

3. Both kinds of cells are very much narrower and especially over the crests of the folds they are longer than in many other regions of the oviduct.

4. In the depressions between the folds the cells are shorter than elsewhere. At the bottom of these depressions there frequently appears to be an accumulation of gland cells. No evidence is at hand to show that these differ physiologically from the other gland cells in this region.

The appearance of the unicellular glands in this region, especially the narrowness of the cells, indicates that possibly they serve a different function than the similar cells in other parts of the oviduct.

The function usually ascribed to the vagina is the production of the outer shell cuticle and a portion, at least, of the coloring matter of the shell. If this assumption is correct these substances must be formed by the unicellular glands described above.

It should also be stated that the walls of the cloaca contain glands. Whether these are concerned with the production of any egg substance or not has not been ascertained.

Discussion.

It may not be out of place to discuss very briefly the possible significance of certain structures which have been described in the fowl's oviduct. It must be remembered, however, that any deduction drawn from the morphology of these structures must be checked and possibly corrected by physiological studies.

As noted on page 404 previous students of oviduct histology have failed to find evidence of glandular structures in the funnel

region. Consequently the funnel has been regarded as an organ for grasping the yolk at the time of ovulation but without any secretory function. In the present study we have been able to show that groups of gland cells occur at the base of the epithelial folds throughout the funnel region. We have further shown that structurally these glands of the funnel are homologous with the tubular glands found in other parts of the oviduct. On the other hand the unicellular glands found in the epithelium of other parts of the duct do *not* occur in the anterior part of the funnel.

The presence of glands in the funnel leads, of course, to the presumption that this region secretes a portion of the substance enveloping the yolk. In the normal hen's egg there is next to yolk a very thin layer of dense albuminous substance. This is known as the *chalaziferous layer* and is continued at the poles of the egg into the familiar structures known as the *chalazae.* It thus appears not unlikely that the chalazae and their corresponding albuminous layer may be secreted in the funnel. I know of no instance in the literature where an egg has been observed in the funnel region before it has entered the albumen secreting portion.

In the albumen secreting portion of the oviduct there are two sets of glands present, viz.: the tubular glands and the unicellular glands of the epithelium. As Pearl and Curtis ('12) have shown, the albumen portion secretes only the *dense* albumen. The outer layer of fluid albumen is not secreted in this region. Apparently both sets of glands take part in the formation of this dense albumen.

The distinctive function of the isthmus is to secrete the shell membrane. A very beautiful demonstration of this act was first described by Coste ('47). Coste described an egg, one end of which had just entered the isthmus. This end of the egg was covered with a thin layer of membrane, while the end still in the albumen portion was naked. This account of membrane formation has several times been confirmed in this laboratory, (Pearl and Curtis, '12). A further very interesting observation is reported by Pearl and Curtis, viz.: that in the so-called albumen portion of the oviduct only about 40 per cent, by weight, of the total albumen is formed. During the passage of the egg through the isthmus it receives in addition to the shell membrane, 10 to 20 per cent more of the total albumen. It is

thus clear that in addition to the shell membranes the glands of the isthmus must also secrete albumen.

The present investigation has not revealed any visible histological difference between the glands of the albumen portion and those of the isthmus. The only differences to be noted are in the smaller volume of the tubular glands and the better development of the muscular layers in the isthmus. So far as the present investigation is concerned, no morphological difference between either set of glands in this region and the corresponding structures in the albumen region have been observed. The one fact of considerable interest, as pointed out below, is the distinct break in the layer of tubular glands between these two regions. The unicellular glands on the other hand are continuous from one region to the other.

Until the work of Pearl and Curtis it was believed that the only substance formed by the uterus was the calcareous shell. These authors have shown that in addition to this, from 30 to 40 per cent, by weight, of the albumen is added to the egg during its sojourn in the uterus. This thin albumen must pass through the shell and the membrane by osmosis.

In the present paper it has been shown (p. 420) that the cells of the tubular glands of the uterus differ in appearance from the corresponding cells in other parts of the oviduct. It seems very probable then that the tubular glands are wholly concerned in the secretion of the shell and that the fluid albumen is secreted by the unicellular glands. The evidence for such an assertion is not complete but it accords with the facts so far observed.

We have then in the uterus an observed differentiation of the cells of the tubular glands which corresponds to the distinct differentiation in function, viz.: shell formation. On the other hand there is no visible differentiation of the unicellular epithelial glands in any portion of the oviduct with the possible exception of the vagina. We further know that two distinct substances are formed in the uterus, viz.: the calcareous shell and a large portion of the fluid albumen. On this basis of fact it is possible to offer the suggestion that throughout the oviduct, with the exception of the vagina, the unicellular glands are concerned in the production of a fluid or thin albumen. On this view the characteristic difference of each region of the

oviduct anterior to the vagina, is due the activity of the tubular glands.

If we extend this idea to the isthmus we may suppose that the membrane is secreted by the tubular glands while the albumen is secreted by the unicellular glands. This is again in accord with the fact that there is an abrupt break in the tubular glands between the albumen portion and the isthmus while the unicellular glands are continuous. The abruptness of the break in the tissue layer at this point corresponds very well with the abruptness in the change of function. Just as soon as the egg crosses the line between the albumen region and the isthmus the formation of membrane begins.

In the albumen region it may be assumed that the tubular glands secrete a very dense albumen while the unicellular glands secrete a more fluid substance which perhaps serves to dilute the former to some extent. There are no observations to support this view but it is not at variance with the facts so far observed.

In the anterior part of the funnel region only the homologues of the tubular glands are present and here we have a dense membranous substance in the chalazae and their accompanying layer.

In the vagina only unicellular epithelial glands are found. These differ somewhat in the form of cell and general appearance from unicellular glands in other parts of the oviduct. These are perhaps concerned in secreting the delicate outer shell cuticle and the coloring matter of the shell.

In conclusion it must again be urged that the deductions in the above paragraphs are offered only as suggestions and that sufficient facts either to prove or disprove them are not at hand

Summary.

1. Two muscular layers, an outer longitudinal and an inner circular layer can be distinguished in all parts of the oviduct.

2. The inner surface of the oviduct is thrown into a number of primary longitudinal ridges. The epithelium over these ridges forms secondary folds. In the uterus the ridges as such are lost and instead there are a number of leaf like folds of the inner surface.

3. Three types of glands are described: (1) Unicellular epithelial glands occurring between the ciliated cells in all parts

of the oviduct except the anterior portion of the funnel. (2) Glandular grooves. These are accumulations of gland cells at the bottom of the grooves between the secondary folds of the epithelium. These are found only in the funnel region. But there they occur well towards the anterior end. The presence of glandular structures in the funnel region has not hitherto been recognized. (3) In all parts of the oviduct between the funnel and the vagina there is a thick layer of glands beneath the epithelium. I have called these *tubular glands.* They consist of long convoluted and branched tubules which open to the lumen of the oviduct by short epithelial ducts. These tubular glands are homologous, structurally at least, with the glandular grooves of the funnel. The tubular glands reach their greatest development in the albumen secreting region. Histologically the unicellular epithelial glands present a similar appearance in all parts of the oviduct except the vagina. In this latter region the cells are longer and much narrower and have a slightly different arrangement than in other parts of the oviduct.

4. The walls of the tubular glands consist of large gland cells which in the albumen portion and the isthmus of a laying hen have small, irregularly shaped, dark staining nuclei which lie well towards the basal ends of the cells. In these two regions the protoplasm of the cells consist of rather coarse granules which vary greatly in size.

5. The line of demarcation between the albumen region and the isthmus is characterized by the absence of these tubular glands in that region. The cells of the tubular glands in the albumen region and in the isthmus present the same histological appearance.

6. In the uterus the cells which form the tubular glands have a somewhat different appearance. The nuclei of these cells are large with regular outlines and are situated near the center of the cells. The protoplasm is very finely granular and is quite different from the coarsely granular condition found in other parts of the oviduct.

7. The tubular glands or any homologous structures are entirely absent from the vagina. Only the unicellular epithelial glands occur here.

8. In the last section of the paper some suggestions are offered as to the probable function of the various glandular structures in the different parts of the oviduct.

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Description of Figures.

Figures 457 to 474 were drawn with the aid of a camera lucida. All figures have been reduced one-half in reproduction. The approximate magnification of each figure, as reduced, is given below.

The following characters have the same meaning in all the figures:

O—Outer muscular layer.

I—Inner muscular layer.

T—Tubular glands.

E—Unicellular epithelial glands.

G—Glandular groove.

First Plate.

Figure 453. Photograph of the funnel region of a functional oviduct showing the expanded lips, the thin walls with their small longitudinal spiral folds and the region of transition to the albumen region. x½.

Figure 454. Photograph of a portion of the albumen secreting region showing the thick longitudinal folds. From the same oviduct as figure 453. x½.

Figure 455. Photograph from the same oviduct showing albumen portion above and isthmus below and also the line of demarcation between these two regions. x½.

Figure 456. Photograph from the same oviduct showing the expanded uterus and the narrow vagina. x½.

Second Plate.

Figure 457. Transverse section of the oviduct wall in the region of the funnel lips. x30.

Figure 458. Transverse section of the oviduct wall in the region of the funnel neck showing primary and secondary folding of the epithelium. x 30.

Figure 459. Transverse section from the region of transition from funnel to albumen portion. Note the presence of a few tubular glands which are entirely absent in the region anterior to this. Muscular layers are not shown. x60.

Figure 460. Section of an epithelial gland from the funnel region. (Cf. figure 462.) x600.

Figure 461. Section of a typical gland from the funnel region. x600.

Figure 462. Transverse section of the oviduct wall from the albumen secreting region. Note the very thick layer of tubular glands. x30.

Third Plate.

Figure 463. Transverse section of the epithelium from the middle of the funnel region. Note pouch like glands from which the ciliated cells are absent except at the center. The epithelium over the ridges has several rows of nuclei. x300.

Figure 464. Section of the epithelium from the region of transition from funnel to albumen portion. This section shows the formation of the tubular glands. x300.

Figure 465. Section from the albumen portion showing the opening of a tubular gland, also the character of the gland cells and of the epithelium. From an actively secreting oviduct. x600.

Figure 466. Gland cells from the tubular glands in the albumen region showing large dark staining granules. x1000.

Figure 467. Section of epithelium from the albumen region showing ciliated cells and unicellular glands. x1000.

Figure 468. Transverse section of the wall of the uterus. x30.

Fourth Plate.

Figure 469. Oblique section through the line of transition from albumen portion to isthmus. Note the absence of all tubular glands along this line. At the left of the figure a portion of the albumen secreting region is shown and at the right a portion of the isthmus. x30.

Fifth Plate.

Figure 470. Section from actively secreting isthmus showing opening of tubules. x600.

Figure 471. Section from isthmus showing gland cells and epithelium after the period of active secretion. x600.

Figure 472. Section of the epithelium and opening of tubular gland from the uterus. x800.

Figure 473. Transverse section through the wall of the vagina. x50.

Figure 474. Section of the epithelium from the vagina showing the unicellular glands. x600.

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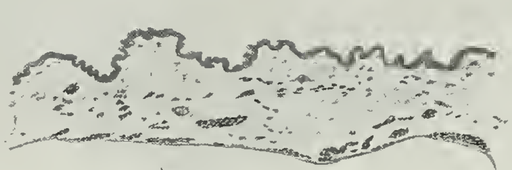
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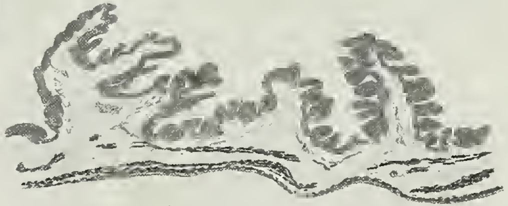
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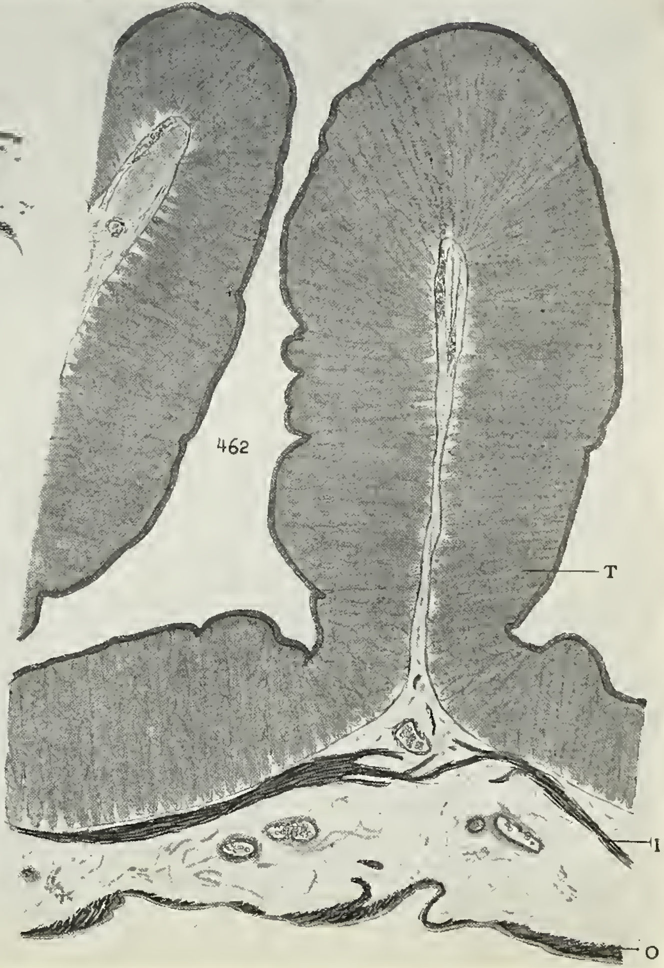

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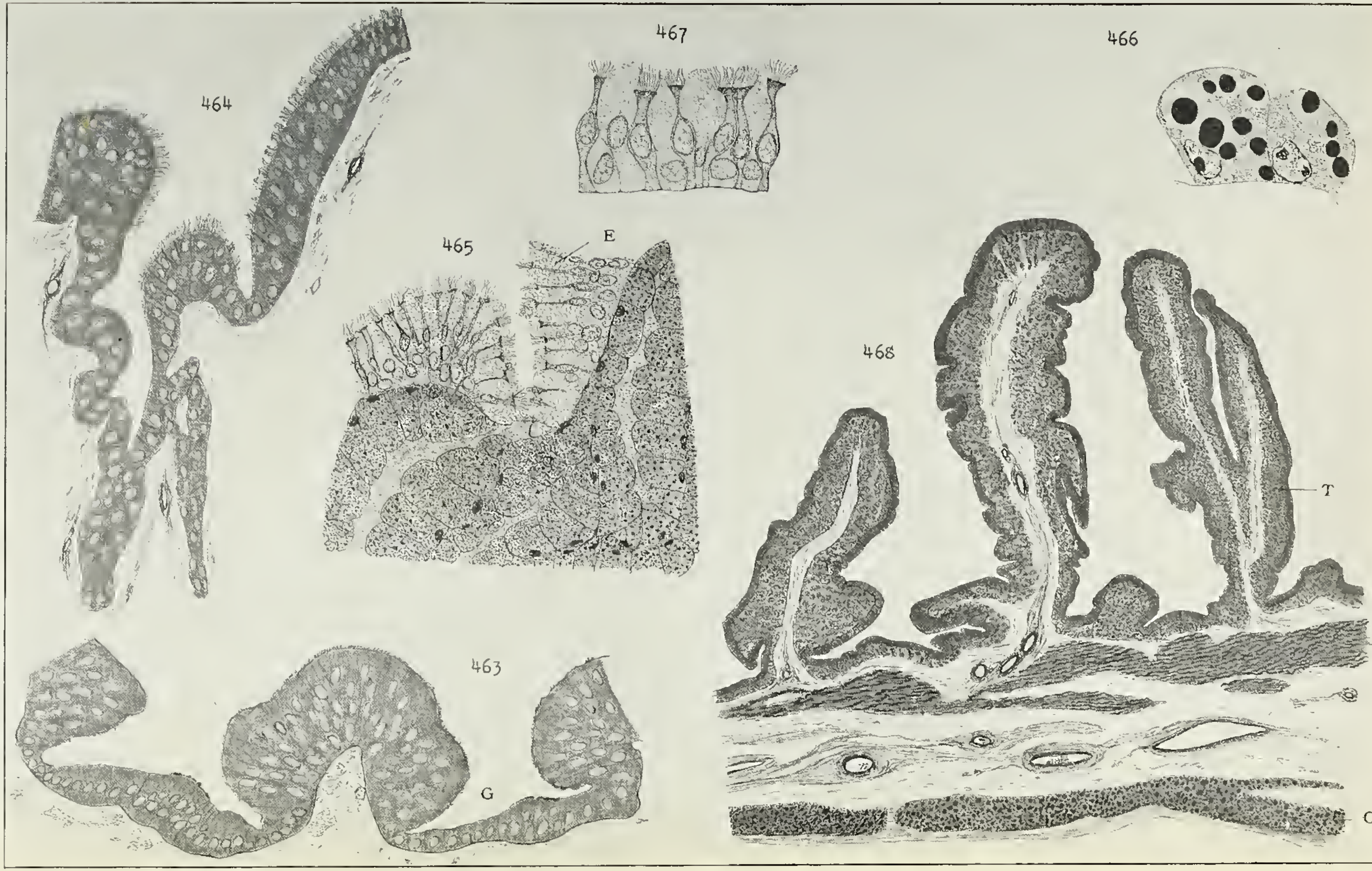
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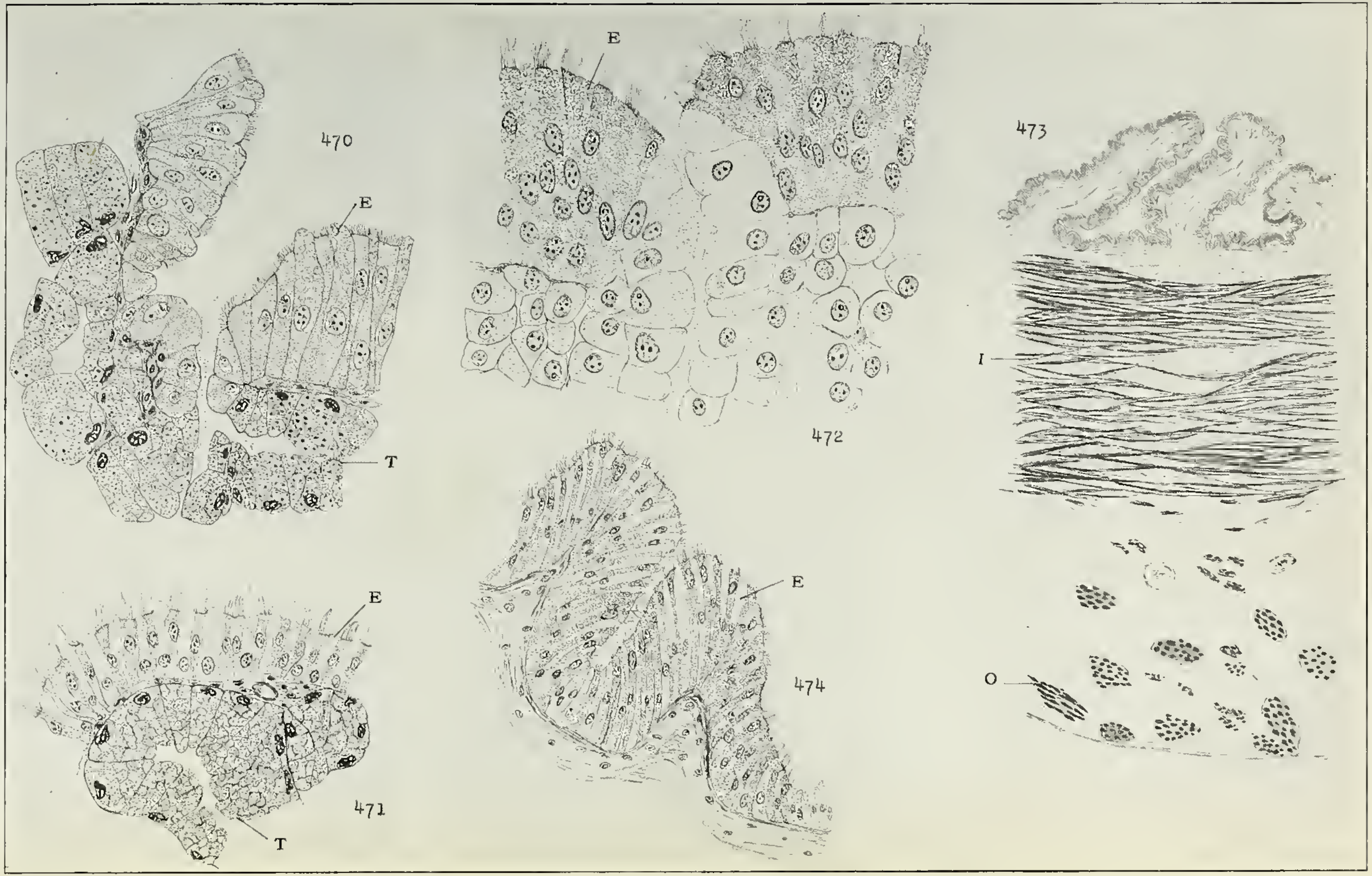
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